



Acute effects of static and proprioceptive neuromuscular facilitation stretching on hamstrings muscle stiffness and range of motion: a randomized cross-over study

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Abstract

This study aimed to compare the acute effects of static stretching (SS) and proprioceptive neuromuscular facilitation (PNF) stretching on hamstrings flexibility and shear modulus. Sixteen recreationally active young volunteers participated in a randomized cross-over study. Participants underwent an aerobic warm-up (WU), followed by either SS or PNF stretching. Range of motion (RoM) during passive straight leg raise and active knee extension, as well as shear modulus of the biceps femoris (BF) and semitendinosus (ST) muscles, were measured at baseline, post-WU, and post-stretching. Both stretching techniques significantly increased RoM, with no differences observed between SS and PNF ($p < 0.001$; $\eta^2 = 0.59\text{--}0.68$). However, only PNF stretching resulted in a significant decrease in BF shear modulus (time \times stretching type interaction: $p = 0.045$; $\eta^2 = 0.19$), indicating reduced muscle stiffness. No changes in ST shear modulus were observed after either stretching technique. There was no significant correlation between changes in RoM and shear modulus, suggesting that the increase in RoM was predominantly due to changes in stretch tolerance rather than mechanical properties of the muscles. These findings suggest that both SS and PNF stretching can effectively improve hamstring flexibility, but PNF stretching may additionally reduce BF muscle stiffness. The study highlights the importance of considering individual muscle-specific responses to stretching techniques and provides insights into the mechanisms underpinning acute increases in RoM.

Keywords Warm-up · Flexibility · Ultrasound · Elastography · Compliance

Introduction

Warm-up (WU) is a well-established practice that includes preparatory exercises performed before the main part of the training session or competition in order to improve neuromuscular performance and reduce the risk of injuries (Bishop 2003; Padua et al. 2019). The mechanisms underlying increased performance include faster neural potential transmission, changes in the force–velocity relationship,

improved oxygen uptake, and increased glycogenolysis, glycolysis, and high-energy phosphate degradation (Bishop 2003; McGowan et al. 2015). In addition, warm-up may improve performance and reduce injury risk by altering musculotendinous stiffness correspondingly to increased muscle temperature (Bishop 2003; Padua et al. 2019). WU usually consists of a general dynamic component, which includes low-intensity movements (e.g., running, cycling), followed by a specific component of slightly higher intensity and sport-specific movements to activate specific muscle groups that will be predominantly loaded further (Behm et al. 2016). A WU routine may also incorporate stretching exercises as a part of a general or specific segment. Especially for muscle groups prone to shortening, such as the hamstrings, adequate stretching can be of a great importance (Díaz-Soler et al. 2015). Tightened hamstrings can lead to muscular imbalances which can contribute to the development of acute and chronic injuries such as patellar tendinopathy and patellofemoral pain (White et al. 2008; Medeiros et al. 2016). Since hamstrings originate in the

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lumbar-pelvic region, low back pain may be related to shortened hamstrings (Hori et al. 2021).

Considering its simplicity, static stretching (SS), which holds muscle in an extended position for a given amount of time, is often performed as a part of the WU routine (Behm et al. 2016; Maeda et al. 2017). Previous studies reported that SS increases range of motion (RoM) acutely (Konrad et al. 2022; Behm et al. 2023a) and in the long term (Behm et al. 2016; Wilke et al. 2020; Konrad et al. 2023). While the effect on all cause injury risk is less clear (Pope et al. 1998; Shrier 1999), there is evidence for a decreased incidence of musculotendinous injuries especially with explosive and change of direction activities (Behm et al. 2016, 2021a, 2023b). Although studies have implied a negative effect of SS on performance (Kay and Blazevich 2012; Simic et al. 2013), such outcomes are only observed after prolonged static stretching (≥ 60 s) (Kay and Blazevich 2012; Behm et al. 2021b) and may be abolished by including dynamic activation exercises within the WU (Reid et al. 2018; Šarabon et al. 2020; Behm et al. 2021b). Another commonly used technique is based on proprioceptive neuromuscular facilitation (PNF) principles (Sharman et al. 2006) and involves a combination of muscle stretching and contraction. One of the most common PNF techniques is hold-relax, wherein an isometric contraction of the stretched muscle is included within the stretching repetition (Sharman et al. 2006; Cayco et al. 2019).

Both static and PNF stretching have been shown to produce similar acute improvements in RoM (Behm et al. 2016; Konrad et al. 2023), including in movements depending on hamstrings flexibility (Borges et al. 2018). The potential underlying mechanisms underpinning the increase in RoM are both peripheral (a decrease in muscle–tendon stiffness) and central (increased stretch tolerance) (Magnusson et al. 1997; Freitas et al. 2018). Muscle stiffness may be inferred from a variety of clinical scales or an assessment of passive joint torque (Marshall et al. 2011; Roots et al. 2022). However, joint torque is potentially influenced by the stiffness of tissues other than muscles and tendons (e.g., ligaments). Recently, ultrasound shear wave elastography (SWE) has emerged as an objective method to assess muscle stiffness (Creze et al. 2018; Roots et al. 2022), allowing researchers to obtain insights into mechanical changes directly within the muscles. Briefly, in SWE, an acoustic radiation impulse is delivered perpendicular to the observed structure, generating shear waves, which travel parallel along the tissue. The stiffness of the tissue determines the velocity of shear waves spreading—the stiffness can, therefore, be reported as an actual velocity of shear wave propagation (m/s) or converted to shear modulus (kPa) (Sigrist et al. 2017).

While the effects of static and PNF stretching on RoM are well documented (Behm et al. 2016; Konrad et al. 2023), it remains unclear if both modalities increase RoM through

the same mechanisms. Several studies have indicated that SS can reduce muscle shear modulus (Zhou et al. 2019; Hirata et al. 2020). Furthermore, Konrad et al. (2017) reported similar effects of static and PNF stretching on ankle dorsiflexion RoM, with both modalities observed to decrease gastrocnemius shear modulus. Yu et al. (2022) reported a significant reduction in biceps femoris (BF) shear modulus after PNF stretching; however, no comparison was made to SS. To our knowledge, no study has compared the effects of static and PNF stretching on hamstrings muscle stiffness. In addition, passive muscle stiffness has been shown to vary among the hamstring muscles (Miyamoto et al. 2020). Furthermore, stiffer muscles may be more susceptible to exhibiting decreased stiffness after stretching (Hirata et al. 2016). Therefore, assessing the responses of individual hamstring muscles to static and PNF stretching would expand our mechanistic understanding of the effects of static and PNF stretching on hamstrings flexibility. Accordingly, this study aimed to compare the outcomes of two stretching techniques (static and PNF hold-relax stretching) on RoM and hamstring muscles (biceps femoris and semitendinosus (ST)) stiffness. We hypothesized that static and PNF stretching improve hamstrings flexibility and reduce shear modulus indicating decreased muscle stiffness. The effects of static and PNF stretching may differ in magnitude, with one possibly leading to more pronounced changes in shear modulus than the other. However, due to the inconclusive results from previous studies, we did not hypothesize which method would be superior. In addition, we hypothesized that there is a correlation between changes in RoM and shear modulus.

Methods

Participants

We used G*Power 3.1 software (Heinrich Heine University, Düsseldorf, Germany) for calculating sample size for a within-factors analysis of variance (ANOVA) (effect size (f) = 0.40; α error = 0.05, and power = 0.80). Medium to large acute effects of SS and PNF stretching on muscle stiffness have been reported before (Konrad et al. 2017; Yu et al. 2022). The effect sizes (f) used for sample size calculation were converted from medium effect size value for ANOVA within the G*Power. The sample size calculation indicated that > 12 participants were needed for the study. Therefore, 16 young recreationally active volunteers participated in the study (nine women; age: 24 ± 1.22 yrs; height: 168.7 ± 6.3 cm; body mass: 61.8 ± 7.4 kg; and seven men; age: 24.3 ± 1.38 yrs; height: 181.4 ± 7.73 cm; body mass: 81.8 ± 16.3 kg). All participants were healthy, without previous or current hamstrings, legs, and torso injuries, myopathy, neuromuscular and neurological disorders. They

were asked to refrain from intensive resistance training for at least two days before the measurements. Although they had not engaged in regular stretching routines over the previous year, they reported performing both dynamic and static stretches as part of their warm-up routines. Each participant was informed about the testing procedure and the purpose of the research. Written consent about voluntary participation was obtained from each participant. The research methods and interventions used are non-invasive, in accordance with the ethical approval of the National Medical Ethics Committee of Republic of Slovenia (No.: 0120-321/2017-4) and in accordance with the Declaration of Helsinki.

Study design

A randomized cross-over study was conducted, with participants visiting the laboratory twice. First, the demographic data were obtained and leg dominance was determined for each participant with the question “If you would shoot a ball on a target, which leg would you use to shoot the ball?” which was found to be reliable in determining leg dominance in bilateral mobilizing tasks (van Melick et al. 2017). Participants rested in a lying position for five minutes, then performed the 5 min of WU by performing step-ups (height = 20 cm) to the beat of the metronome (120 bps). This was followed by either SS or PNF, depending on the visit. The order of stretching intervention was randomly determined for each participant by the sealed opaque envelope method. On each testing day, RoM and muscle stiffness were measured (before warm-up, after the aerobic part of the WU, and after static or PNF stretching). The second intervention was performed for each participant at approximately the same time as the first to avoid the influence of circadian rhythms with at least 24 h rest period between to avoid the impact of the previous intervention. Both were carried out in the same air-conditioned room with a temperature between 22 and 23 °C.

Stretching interventions

Both stretching techniques were carried out with the participant in a supine position on the examination table and performing hip flexion with knee extended (Fig. 1). The intensity of stretching was determined by the participants' initial sensation of mild discomfort. In the SS technique, the hamstrings were passively stretched by the examiner, with the knee extended, for 30 s. Based on previous studies (Barroso et al. 2012; Kay et al. 2015; Oliveira et al. 2018), the PNF was performed with the hold-relax technique as follows: hamstrings were passively stretched with a straight leg raise for 5 s, followed by a 5-s submaximal isometric contraction at 50% of perceived maximal effort against a manual resistance applied on the distal part of the tibia and

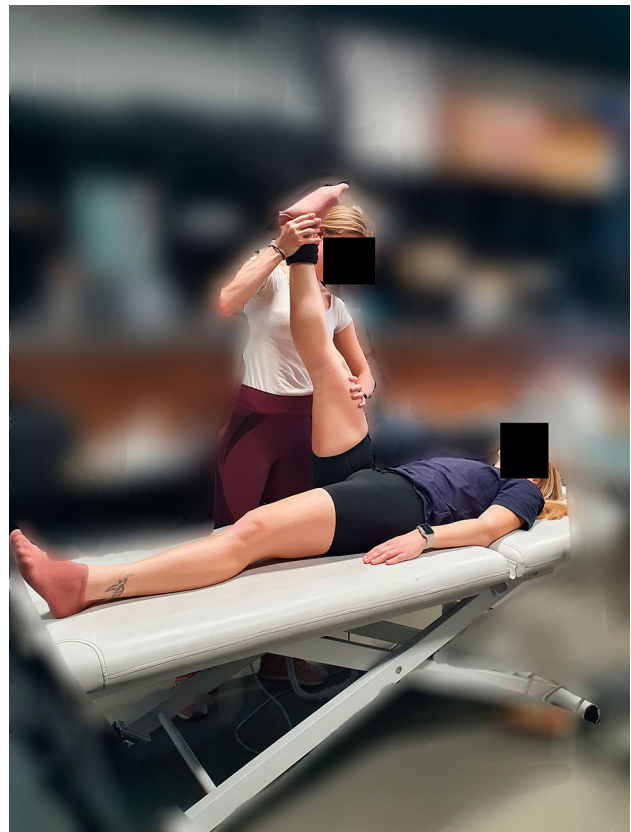


Fig. 1 Position and movement used in the stretching intervention

then stretched for additional 20 s, resulting in the same total duration as in SS. Three sets of each technique were performed with a 30-s rest period between each set.

Measurement procedures

Muscle stiffness was measured on two hamstring muscles—BF and ST, using an ultrasound system (Resona 7, Midray, Shenzhen, China). The quantitative SWE method, presuming a muscle tissue density of 1000 kg/m³ was used, and the results were expressed as shear modulus (kPa). A medium-sized linear probe (L11-3U, Midray, Shenzhen, China) with a water-soluble hypoallergenic ultrasound gel (AquaUltra Basic—Ultragel, Budapest, Hungary) was placed over the muscle belly of BF and ST. The probe was placed at 50% on the line between the ischial tuberosity and the lateral (BF) and medial (ST) epicondyle of the tibia, following the SENIAM recommendations for EMG recordings (Hermens et al. 2000). The region of interest was 1×1 cm, and its depth was determined individually, ensuring to only include muscle tissue (Fig. 2). The depth was recorded and used on a subsequent visit. The probe was oriented to follow the line of the muscle fascicles, which was determined in B-mode images before

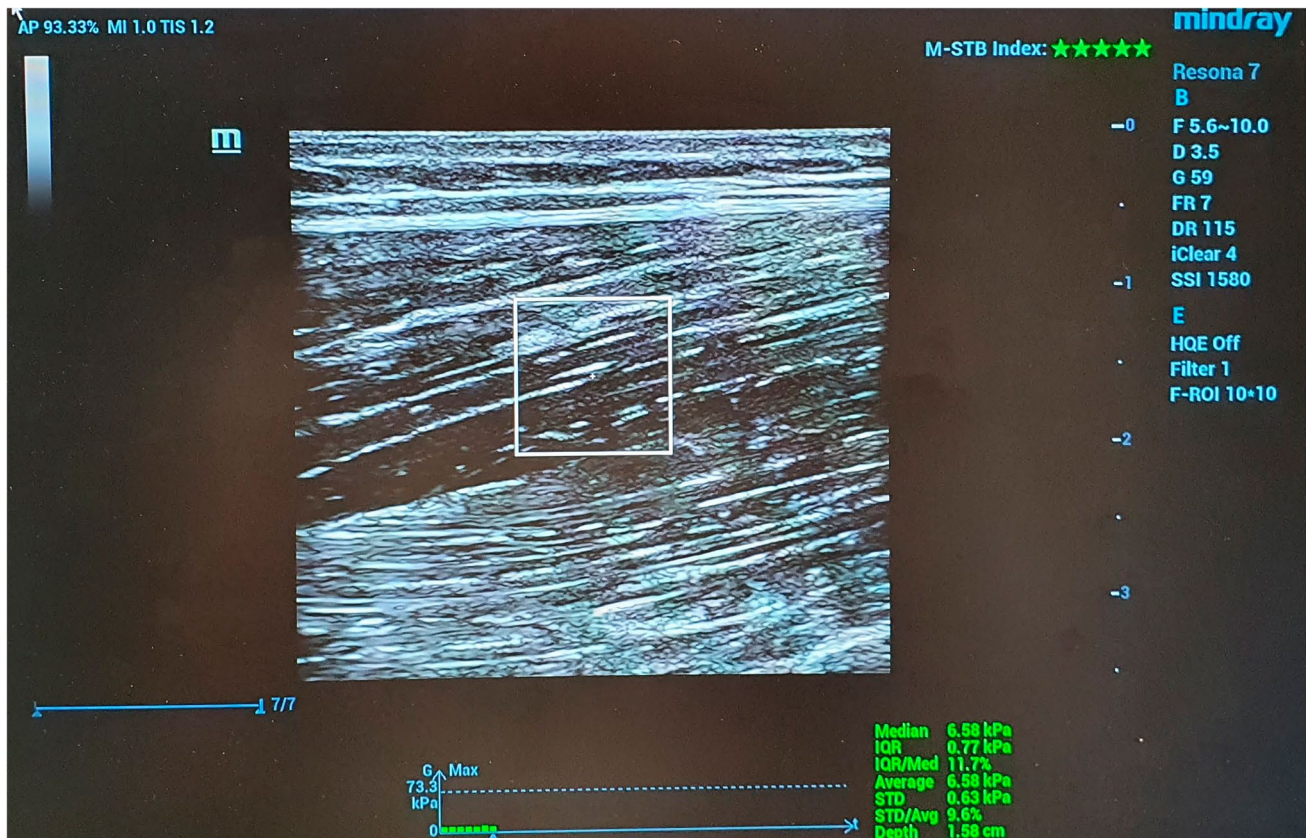


Fig. 2 A snapshot of ultrasound measurements

the stiffness recordings. The stiffness was measured twice after each time point (before warm-up, after the aerobic part of the warm-up, and after stretching) and was determined as the mean of eight consecutive measurements (separated by 1 s), which is the maximum storage capacity of the device.

Two tests were performed for RoM assessment—passive straight leg raise (SLR) and active knee extension (AKE). Both tests were performed in a supine position on the examination table using a digital inclinometer, attached to the tibia just above the malleolus. During the SLR test, the participants were instructed to remain relaxed and to not produce any voluntary muscle contractions. From starting point on the table (0°), the examiner gently raised the participant's testing leg by flexing the hip with the passively provided knee extension to the point where the participant reported mild discomfort. The other examiner recorded the value, while the non-tested leg rested straight on the table. In the AKE test, participants were instructed to maximally extend the knee and hold the position while having the hip passively flexed at 90° . The examiner placed the leg into 90° hip flexion and 90° knee flexion (starting position), while the other examiner recorded RoM after maximal active knee extension. The non-tested leg was lying flat on the table. Both

RoM assessments were performed twice at all time points (baseline, post-WU, and post-stretching).

Statistical analysis

SPSS statistical software was used (IMB, Armonk, NY, USA). Data are presented as means and standard deviations. The normality of the data distribution was verified with Shapiro–Wilk test and visual inspection of histograms and Q–Q plots. General linear model repeated measures 2-way ANOVA (2×3) with stretching type (static, PNF) and time (baseline, post-WU and post-stretching) as within-participant factors was used, with post-hoc *t* test performed in case of significant main effects and interactions. The effect sizes were expressed as partial η^2 and interpreted as trivial (< 0.02), small (0.02–0.13), moderate (0.14–0.26) and large (> 0.26) (Bakeman 2005). Baseline differences between visits were examined with paired *t*-tests. The associations between changes in RoM and changes in shear modulus were examined with Pearson's correlation coefficient (*r*). The correlations were interpreted as negligible ($r < 0.1$), weak ($r = 0.1$ –0.4), moderate ($r = 0.4$ –0.7), strong ($r = 0.7$ –0.9) and very strong ($r = 0.9$). The threshold for statistical significance was set at $\alpha < 0.05$.

Results

Baseline values

There were no baseline differences before the two stretching conditions for SLR test (PNF: $85.6 \pm 11.9^\circ$; static: $84.0 \pm 11.9^\circ$; $p=0.532$), AKE test (PNF: $74.2 \pm 10.2^\circ$; static: $74.5 \pm 11.7^\circ$; $p=0.926$), BF shear modulus (PNF: 9.97 ± 1.98 kPa; static: 10.11 ± 2.05 kPa; $p=0.809$) and ST shear modulus (PNF: 11.21 ± 3.10 kPa; static: 11.82 ± 3.09 kPa; $p=0.509$).

Effects of WU and stretching on RoM

For RoM during SLR test, there was a large effect of time ($F=32.5$; $p<0.001$; $\eta^2=0.68$), but no effect of stretching type ($F=0.19$; $p=0.665$), nor time \times stretching type interaction ($F=0.58$; $p=0.564$). Post-hoc tests indicated a statistically significant increase from baseline to post-WU ($p=0.004$), from baseline to post-stretching ($p<0.001$) and from post-WU to post-stretching ($p=0.001$) (Fig. 3A). Similarly for AKE test, we observed a large effect of time ($F=22.2$; $p<0.001$; $\eta^2=0.59$), but no effect of stretching type ($F=0.01$; $p=0.936$), nor time \times stretching type interaction ($F=0.60$; $p=0.554$). Post-hoc test again indicated a statistically significant increase from baseline to post-WU ($p=0.020$), from baseline to post-stretching ($p<0.001$) and from post-WU to post-stretching ($p<0.001$) (Fig. 3B). In summary, RoM increased with WU and further with stretching, with similar effects regardless of the stretching type.

Effects of WU and stretching on shear modulus

For BF shear modulus, there were no main effects of stretching type ($F=0.96$; $p=0.343$) and time ($F=1.95$; $p=0.159$), but there was a statistically significant time \times stretching type interaction ($F=3.45$; $p=0.045$; $\eta^2=0.19$). Separate analyses for each stretching type indicated a statistically significant effect of time in PNF condition ($F=7.36$; $p=0.003$; $\eta^2=0.33$), but not in the static stretching condition ($F=1.07$; $p=0.353$). In PNF condition, the post-hoc tests indicated no difference between baseline and post-WU ($p=0.135$) or between baseline and post-stretching ($p=0.208$), but there was a statistically significant decrease between post-WU and post-stretching ($p=0.013$) (Fig. 4A). For ST shear modulus, there were no effects of time ($F=0.61$; $p=0.554$) or stretching type ($F=0.31$; $p=0.583$), nor time \times stretching type interaction ($F=0.22$; $p=0.801$) (Fig. 4B).

Associations between changes in RoM and changes in shear modulus

In both conditions, there were no statistically significant correlations between relative (%) changes in RoM tests and changes in shear modulus for either muscle ($r=-0.19$ – 0.38 ; all $p \geq 0.142$).

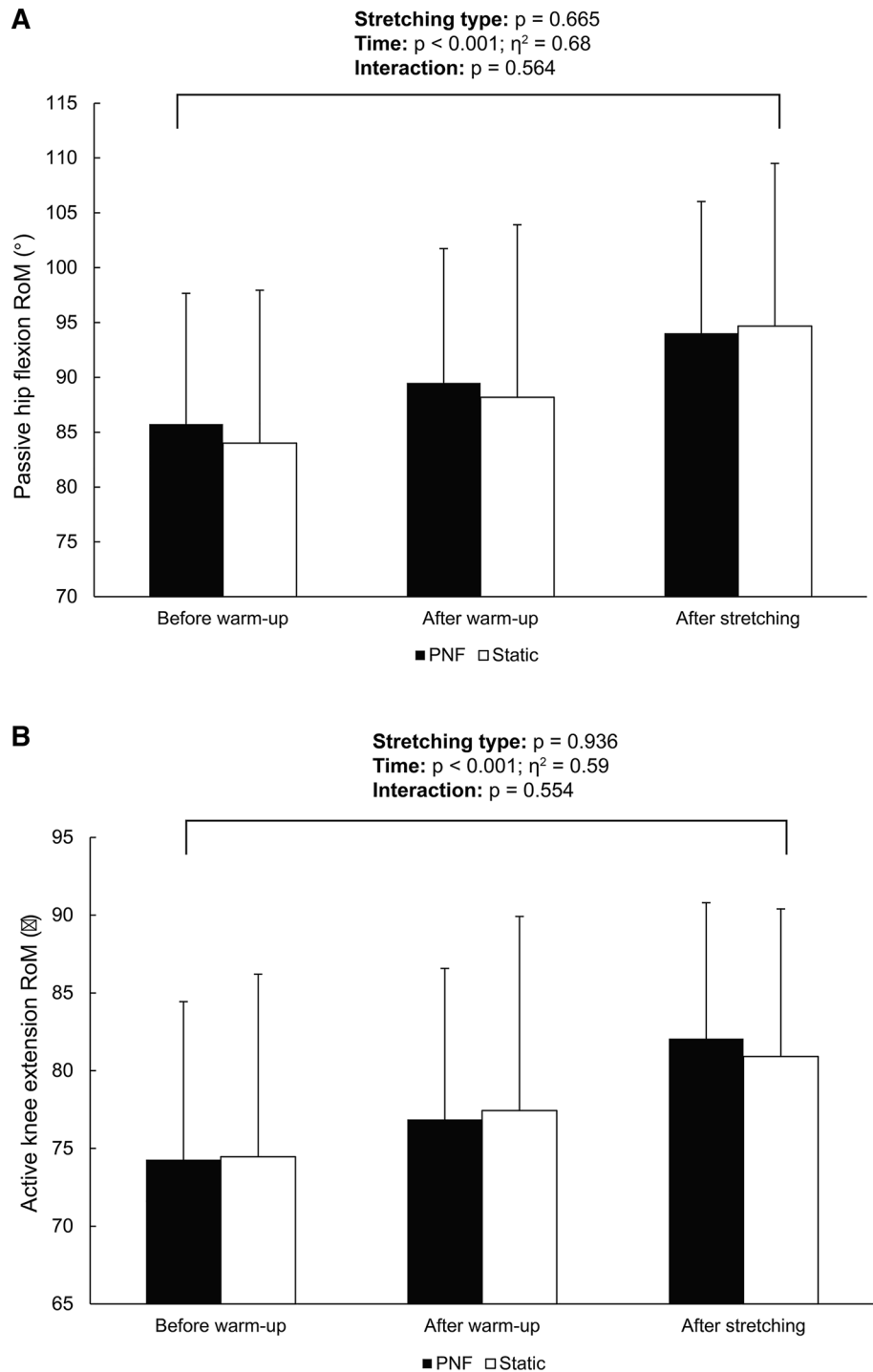
Discussion

The aim of this study was to compare the acute effects of SS and PNF stretching (hold-relax) stretching on flexibility and stiffness (shear modulus) of the hamstring muscles. The results showed an increase in active and passive RoM after WU, with a further increase after both stretching techniques. We did not detect a change in shear modulus for BF or ST after SS at any time point, but there was a significant decrease in shear modulus for BF after PNF stretching. In addition, there was no correlation between changes in RoM and shear modulus. Our results suggest that different mechanisms may underlie the increases in RoM after SS and PNF stretching.

There was a significant improvement in active (AKE) and passive (SLR) RoM after the aerobic WU. This was expected because aerobic activity increases muscle temperature and decreases muscle viscosity (thixotropic effects), leading to increased RoM (Bishop 2003; Behm 2018). When WU transitioned to stretching, both active and passive RoM continued to increase, regardless of the stretching method. Similar increases in active RoM acutely after SS and PNF have been reported previously (Funk et al. 2003; Ford and McChesney 2007; Puenteadura et al. 2011; Maddigan et al. 2012; Lim et al. 2014; Konrad et al. 2017; Nakamura et al. 2021), although some authors found PNF to be more effective than SS (O'Hara et al. 2011). Consistent with our findings, Maddigan et al. (2012) and Mani et al. (2021) reported that there was no difference in the increase in passive hip flexion RoM between PNF and SS. These findings are also in accord with recent meta-analyses on the acute and chronic effects of stretching overall (Behm et al. 2023a; Konrad et al. 2023) and specifically with the hamstrings (Borges et al. 2018) indicating no significant differences in ROM between SS and PNF.

Shear modulus for BF decreased significantly after PNF stretching but not after SS. Previous studies reported no changes in shear modulus of plantar flexors after a short bout of SS (Sato et al. 2020; Konrad and Tilp 2020), although there was an increase in RoM. Reduced muscle stiffness after SS was generally observed only when the stretch lasted at least 2 min (Akagi and Takahashi 2013; Nakamura et al. 2019). However, Nakamura et al. (2021) observed a decrease in muscle stiffness in older adults after four sets of 20 s of

Fig. 3 The effects of warm-up and stretching on RoM, as assessed by passive hip flexion (A) and active knee extension (B) tests

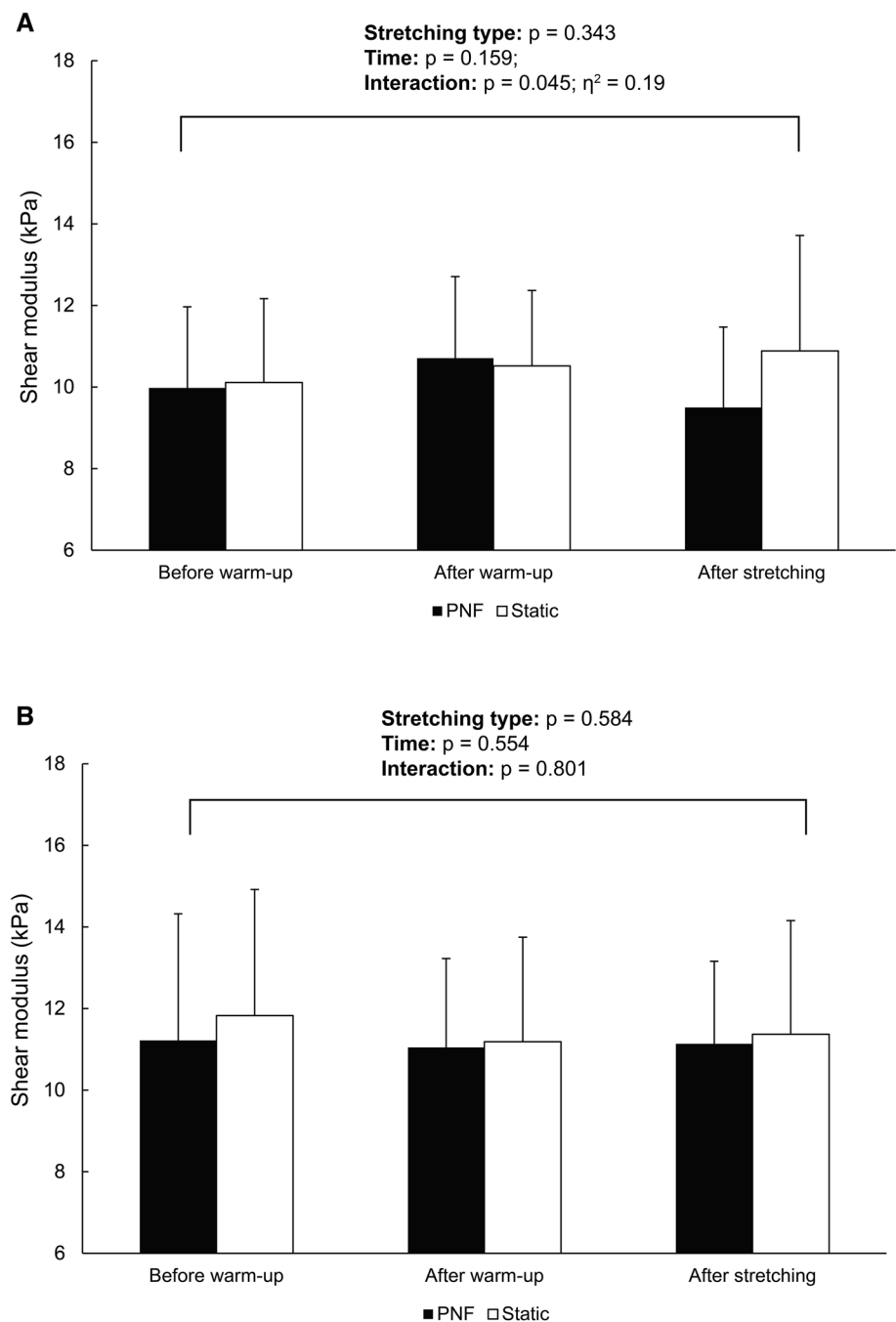


SS. These discrepancies may be explained by age-related physiological differences between young and older individuals and by studies targeting different muscle groups. Overall, the results suggest that the SS protocol used in the current study (90 s total SS) may not be a sufficient stimulus to reduce muscle stiffness. Therefore, our study confirms the findings of Magnusson et al. (1997) and Konrad and Tilp (2014) that the increase in RoM after a brief exposure to SS

results predominantly from a change in central mechanisms such as increased stretch tolerance.

In contrast to SS, PNF stretching caused a statistically significant decrease in shear modulus. To our knowledge, this is the first study to examine the acute effects of PNF stretching on hamstrings shear modulus. Consistent with our findings, Kay et al. (2015) reported a significantly greater decrease in triceps surae muscle–tendon

Fig. 4 The effects of warm-up and stretching on shear modulus of biceps femoris (A) and semitendinosus (B)



stiffness after PNF compared to SS. Specifically for the hamstring muscles, Yu et al. (2022) reported a significant reduction in shear modulus after PNF stretching; however, no comparison was made to SS. As mentioned previously, the increase in RoM after stretching can be explained by peripheral (changes in intramuscular connective tissue due to the applied stretching force) and central processes (increased stretch tolerance) (Miyamoto et al. 2018; Yu et al. 2022). PNF stretching has been suggested to utilize autogenic inhibition, in which the Golgi tendon organs sense increased force within the tendon and send

inhibitory signals to the muscle, allowing for additional stretch (Sharman et al. 2006). However, Golgi tendon organs are reported to be relatively insensitive to stretch-induced tension (Edin and Vallbo 1990) and furthermore, this reflex inhibition diminishes rapidly (within seconds) following the cessation of PNF stretching (Behm 2018). In addition, PNF involves SS and muscle activation, the latter possibly leading to an increase in muscle temperature, thereby decreasing viscoelastic resistance (Opplert and Babault 2018), which might explain decreased shear modulus. Further research is needed to monitor the change in

muscle temperature during WU and PNF to more precisely determine the underlying mechanisms of PNF reducing muscle stiffness.

Interestingly, BF but not ST shear modulus was decreased after PNF stretching. Muscle-specific responses to stretching have been reported before; for instance, studies by Hirata et al. (2016, 2020) indicated that stiffer muscles within the triceps surae complex are more likely to exhibit decreases in shear modulus after stretching. Simpson et al. (2017) also reported different responses in muscle fascicle lengths changes between lateral and medial heads of the gastrocnemius after 6 weeks of stretching, which could be attributed to differences in muscle architecture. Therefore, we can speculate that BF was stretched to a greater extent than ST in our study. Indeed, while ST and BF have similar slack angles, elastography studies have indicated higher increase in tension in BF than ST during stretching maneuver (Le Sant et al. 2015), similar to the one used in the current study. However, it should also be noted that hamstring muscles (both ST and BF) architecture is not uniform along the muscle regions (Kellis et al. 2010) and that shear modulus values are also region-specific (Vaz et al. 2021). Therefore, assessing multiple muscle regions should be considered in future studies.

We found no association between changes in RoM and shear modulus. This suggests that RoM was predominantly increased due to increased stretch tolerance, without notable changes in mechanical muscle properties. While BF shear modulus was decreased, several other muscles and other soft tissues contribute to hip RoM, which could explain the lack of correlation between BF shear modulus and RoM. There is a need for further research to establish the correlation between RoM and shear modulus changes. It would also be insightful to assess the relative contributions of the fascicle and free-tendon elongation to the total lengthening of the muscle–tendon unit during static and PNF stretching, and subsequently explore both muscle and tendon stiffness changes, as well as investigate changes in muscle architecture as a possible confounder (Lévenéz et al. 2023).

Some limitations of the study must be acknowledged. The researchers were not blinded regarding the stretching technique performed. The performance of AKE and SLR tests might have influenced the gain in flexibility and RoM. However, the scope of testing was the same for SS and the PNF conditions. The testing sample included young adults; therefore, our results are not generally applicable to the general population or to the long-term effects. While many similar prior studies focused on the gastrocnemius muscle, our emphasis on the hamstrings (despite some shared characteristics like their pennate structure, biarticular nature, and a high proportion of Type II muscle fibers) means that our results are not entirely comparable with previous work. More research should be done to clarify the optimal duration and type of stretching to induce

changes in muscle stiffness in various populations. For PNF specifically, various methods with different contraction and stretch durations and number of cycles have been used. More research is essential to determine how these variables impact the effect of PNF stretching on the shear modulus.

Conclusions

The present study investigated the acute effects of SS and PNF stretching on hamstrings flexibility and shear modulus. Our findings showed that both stretching techniques significantly increased active and passive RoM, with similar effects observed regardless of the stretching type. However, only PNF stretching resulted in a significant decrease in shear modulus in the BF muscle. The results suggest that the acute increase in RoM after both SS and PNF stretching may be attributed predominantly to central mechanisms, such as improved stretch tolerance. PNF stretching, in addition to SS, may also cause reduced muscle stiffness. Interestingly, only BF (but not ST) showed a decrease in shear modulus after PNF stretching. This may be related to variations in muscle architecture and the specific tension experienced by each muscle during the stretching maneuver. These findings provide insights into the mechanisms underpinning acute increases in RoM after different stretching techniques.

Author contributions DB and ZK conceptualized the idea. PZ, AJ, and KK recruited the subjects and organized the measurement sessions. PZ, KK and AJ carried out the measurements. PZ and AJ analyzed the data. ZK and DB were overseeing the measurement procedures and administration. PZ wrote the first draft of the manuscript. All the authors worked on finalizing the manuscript.

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Data availability All collected data are included in the manuscript. Raw data are available upon reasonable request to the corresponding author.

Declarations

Conflict of interest The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The research methods and interventions used are non-invasive, in accordance with the ethical approval of the National Medical Ethics Committee of Republic of Slovenia; No.: 0120-321/2017-4) and in accordance with the Declaration of Helsinki.

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